IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Valery Kh. ZHILOV, et al.

Group Art Unit: 1623

Serial No.: 10/567,113

Examiner: Patrick T. Lewis

Filed: February 3, 2006

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For: USE OF CYCLIC BIOISOSTERS OF PURINE SYSTEM DERIVATIVES FOR TREATING DISEASES PRODUCED BY DISORDERS OF NITERERGIC AND DOPAMINERGIC SYSTEM

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450 Alexandria,
VA 22313-1450

Sir:

This is a Declaration under 37 C.F.R. §1.132 by Valery Kh. Zhilov in the above-identified application.

I, the undersigned, Valery Kh. Zhilov, declare and state that:

1.I am a co-inventor of the subject patent application having serial no. 10/567,113.

2.I have got a professional experience as an researcher and maker in the area of Pharmaceutics.

3.I have read and understand U.S. Patent Application Serial No. 10/567,113, entitled "USE OF CYCLIC BIOISOSTERS OF PURINE SYSTEM DERIVATIVES FOR

TREATING DISEASES PRODUCED BY DISORDERS OF NITERERGIC AND DOPAMINERGIC SYSTEM," and I submit this Declaration in its support.

4.I have read and understand the June 3, 2009 Final Official Action issued in the above-identified case.

5.I have read and understand the publications of Yurugi et al. *Chem. Pharm. Bull.* 20:1513-1521 (**1972**), International Patent Application No. WO2002/09681 to Zhilov et al., of which I am a co-inventor, and Goldenberg, *Clinical Therapeutics* 20(6):1033-1048 (**1998**), cited by the Examiner.

6.In particular, I understand that in the June 3, 2009 Final Official Action, the Examiner rejected claims 28, 29 and 33, alleging that the claims, drawn to a method of treating diseases caused by disorders of nitrergic system and/or dopaminergic system, are made obvious by Yurugi drawn to a method of synthesizing, *inter alia*, a 2-Substituted-5,6,7,8-tetrahydropyrimido[4,5-d]pyridazine-5,8-dione that may or may not have a hypotensive activity in view of Zhilov that describes the pharmacologically adequate salts, and Goldenberg that describes that some vasodilators may be used in treating the erectile dysfunction. As a person skilled in the art, I respectfully disagree with this rejection and submit that the compounds identified in claims 28 and 29 have the unexpected results in treating diseases caused by disorders of nitrergic system and/or dopaminergic system, which has been for the first time identified by the present inventors.

7.As one of the co-inventors of the instant application, I hereby submit that we conducted a study to ascertain the effects of the compounds according to the instant invention on impairments of sexual function associated with disorders of dopaminergic system. One of the

mechanisms regulating the sexual function of mammals is associated with the functioning of the dopaminergic system of the brain. It is well known in the art that a non-selective D1/D2 agonist apomorphine in low doses causes penile erection in rodents (Giuliano. F., Allard J., "Dopamine and male sexual function," *Eur. Urol.*, 2001, 40 (6), 601-608; Giuliano F., Allard, J., Rampin, O. et. al., "Pro-erectile effect of systemic apomorphine: existence of a spinal site action," *J. Urol.*, 2002, 167 (:1), 402-406; Brien S.E., Smallegange C., Gofton W.T., et. al., "Development of a rat model of sexual performance anxiety: effect of behavioral and pharmacological hyperadrenergic stimulation on apomorphine-induced erections," *Int. J. Impot. Res.*, 2002, 14 (2), 107-115). In light of these findings, we studied the effects of the test compounds as disclosed in the above-identified application on apomorphine-dependent erection in rats and humans (males).

8.We used nine groups of adult male Wistar rats weighing 350-450 g in the experiment that were maintained at the normal (not inverted) light cycle. To study the effect of the test compounds on the sexual function, low-potency animals with a single erection were selected. Control group 1 (n=7) was composed of animals not injected with the test compounds. The animals of groups 2-10 (n = 7) were injected with one of the test compounds selected from the group of compounds 2, 8, 9, 15, 19, 25, 31, 36, and for comparison, a PDE5 inhibitor, Tadalafil (Brand Name: Cialis®), which is presently marketed by Eli Lilly and Company to treat the erectile dysfunction (ED).

9.Apomorphine was dissolved in 0.1% aqueous solution of ascorbic acid and then was injected to all animal subcutaneously at a dose of 0.1 mg/kg during 24-28 hours after the last. injection of the test compounds. The monitoring of sexual activity was made individually for each animal immediately after the administration of apomorphine; the monitoring time was

20 minutes. The following indices were registered: the time of beginning of the first erections, time intervals between the erections, and the number of erections for the entire period of observation. The statistical analysis of the results was made using T-test and x-square test. The results are given in Table 1 as means ± standard deviation (error).

TABLE 1: The effect of the compounds according to the invention on disorders of sexual function of rats

Group (experimental conditions)	**Latent Period of first erection, (minutes)**	**Number of erection for the entire period of observation**	**Time between 1st and 2nd erections, (minutes)**	**Time, between 3rd and. 2nd erections, (minutes)**
No.1 (Control apomorphine)	6.63±0.99	2.00±0.37	4.60±0.53	5.90±0.87 •
Nos. 2-3 (Compound 2 or 8 + apomorphine)	5.21±0.30 P=0.1	3.13±0.35 P<0.05	3.94±0.60	.5:52±1.14 •
Nos. 4-5 (Compound 9 or :1.5 + apomorphine)	5.09±0.27 P<0,1	3.24±0.39 P<0.05	3.87±0.59	• 5.19±0.78
Nos. 6-7 (Compound 19 or 25 + apomorphine)	5.24±0.19 p<0.1	3.29±0.27 P<0.05	3.75±0.64	'5.12±0.87
Nos. 8-9 (Compound 31 or 36 + apomorphine)	5.1.2±0.24 P<0.1	3.32±0.31 121.0.05	3.34±0.69	5.12±1.01 .
Nos. 10 Tadalafil (Cialis)	**6.14±0.22 p<0.1**	**2.50±0.17**	**4.2±0.23**	**5.75±0.18**

10. Two group of males (men) (n = 5) were injected intramuscularly with one of the test compounds: sodium salt of 5-amino-benzo[d]-3H-pyridazine-1,4-dione or potassium salt of

6-amino-benzo[d]-3H-pyridazine-1,4-dione. A third group of males (n = 5) were injected intramuscularly with Tadalafil (Cialis®). The results. are given in Table 2.

TABLE 2: The therapeutic effect of the compounds according to the invention on disorders of sexual function of men

Group (experimental conditions)	**Latent period of first erection, (minutes)**	**Duration of therapy action, hours**	**Side effects**
sodium salt of 5-amino-benzo[d]-3pHyr-i dazine-1,4-di	.10.12+0.10 P<0.1	72 h	unnoticed
potassium salt of 6-amino-benzoic*. 3H-pyridazine-1.,4-dione	8.09±0.22 P<0.1	67 h	unnoticed
Tadalafil (Cialis)	**15.2±0.51 P<0.1**	**36 h**	**Headache, dyspepsia, muscle pain .**

11.From the results of the investigation given in Tables 1 and 2, it is readily ascertainable that the test compounds according to the invention have reliably increased the number of erections of the animals (more than 1.5 times) and demonstrated a statistically significant trend in decreasing the latent period of the first erection by 1.3 times.

12.As one of the co-inventors of the instant application, I hereby submit that the beneficial effects of the compounds according to the invention on the sexual function of rats and male humans has been established. Since the used model involves certain cerebral mechanisms, the mechanism of action of the compounds according to the invention is associated with its effect on the dopaminergic system of the brain, in particular, by correcting the dysfunction of D1/D2 dopamine receptors. The obtained results also demonstrate that the compounds according to the

instant invention can also be used for correction of numerous pathologies of the nervous system associated with the dysfunction of the nitrergic system and/or dopaminergic systems.

13.It is my experience and my opinion, as one skilled in the art of Pharmacological Sciences that in light of the unexpected results of the present invention, a scientist in the relevant field of art armed with the disclosure provided by the Yurugi reference in conjunction with the Zhilov reference and the Goldenberg reference would not have the knowledge and/or any suggestion at the time of filing of the instant invention to deduce that the compounds disclosed in the instant application are effective in treating the dysfunctions of the nitrergic system and/or dopaminergic systems.

14.I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Respectfully submitted,

Date : 02.04.2010

Valery Kh. Zhilov